

Attorney Docket No.: DC-0199
Inventors: Cheung et al.
Serial No.: 10/043,539
Filing Date: January 11, 2002
Page 2

In the Specification:

Please replace the paragraph beginning at page 30, line 8, ^{6, Sec 2/21/02} with the following rewritten paragraph:

--Cloning and sequence analysis of the *sarR* gene. To clone the gene encoding SarR, we blotted the ~12 kDa protein onto a PVDF membrane for N-terminal sequencing. The first 14 amino acids were X(K)IND(I)NDLVNA(S/T)F, (~~Seq.~~ SEQ. ID NO.:8) with X being an unknown residue while those residues in parenthesis carried a putative assignment. In search the databank of the partially released *S. aureus* genome (~~www.tiger.org~~), we obtained a partial ORF of 47 amino acid ~~sequence~~ acids that corresponds to the N-terminal sequence of the ~12 kDa protein. By using two degenerate oligonucleotides of 30-nt each, a 141-bp fragment was amplified to probe a chromosomal digest of *S. aureus* strain RN6390, thus allowing identification of a ~4 kb *Cla*I hybridizing fragment. A plasmid DNA library containing ~3.5 kb *Cla*I fragments constructed in pACYC177 (26) was then screened with the 141-bp PCR-generated probe. A positive clone (pALC1361) yielding a ~4-kb insert at the *Cla*I site of pACYC177 vector was identified. In determining the sequence of the insert, and comparing the insert sequence with that of the 141-bp probe, the DNA sequence of the putative gene *sarR* was obtained (Fig. 1B) (GenBank accession #AF207701). The predicted SarR protein contains 115 amino acids, with a predominance of charged residues (34%) and a predicted molecular size of 13,689 daltons. The *sarR* gene has a putative shine Dalgarno sequence (AGGAGTGG) (SEQ. ID NO:9) lying 7-bp upstream of the translation star, with typical initiation (ATG) and termination codons (TAA).

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Please replace the paragraph beginning at page ~~2~~⁴, line ~~27~~¹¹, 2/21/07 JOC
with the following rewritten paragraph:

-- The present invention provides a new genetic locus of *S.aureus* and other bacteria. The gene at this locus is referred to herein as *sarR* (staphylococcal accessory regulatory protein R). The *sarR* gene is involved in the regulation and expression of virulence determinants in *S.aureus* and other bacteria. --

Please replace the paragraph beginning at page ~~22~~²⁷, line ~~31~~³, JOC 2/21/07
with the following rewritten paragraph:

-- The activities of *sarA* promoter fragments linked to the *gfp_{uvr}* reporter gene in RN6390 and its isogenic *sarR* mutant were assayed by flow cytometry. Bacterial cell suspensions obtained at different parts of the growth cycle were analyzed in a ~~FACscan~~ FACSCAN cytometer (Becton Dickinson, Franklin Lakes, NJ). After filtering bacterial samples through a ~~5µm~~ 5 micron filter to remove large aggregates, bacteria were detected by side scatter data as described by Russo-Marie et al. (56). Fluorescence and side scatter data were collected with logarithmic amplifiers. The fluorescence data were reported in fluorescence units as specified by the instrument (~~FACscan~~ FACSCAN cytometer).--

Please replace the paragraph beginning at page ~~26~~³¹, line ~~1~~¹⁵, JOC 2/21/07
with the following rewritten paragraph:

-- **Over-expression of SarR and production of monoclonal antibodies:** To obtain a large amount of *SarR*, the *sarR* gene was cloned into pET11b and the gene product was over-expressed under an IPTG-inducible promoter in *E.coli* BL21. The expression,

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In the Specification:

Please replace the paragraph beginning at page 1, line 8, with the following rewritten paragraph:

-- The present invention relates generally to the field of molecular biology. More particularly, certain embodiments concern methods and compositions comprising DNA segments and protein derived from ~~Staphylococcus aureus~~ Staphylococcus aureus and other bacterial species. The present invention also relates to the three-dimensional structure of proteins derived from *S.aureus* and other bacterial species and methods of identifying and developing pharmaceuticals using, among other things, drug screening assays.--

Please replace the paragraph beginning at page 2, line 13, with the following rewritten paragraph:

-- *S.aureus* can cause a wide spectrum of infections ranging from superficial abscesses, pneumonia and endocarditis to sepsis (4). The ability of *S.aureus* to cause a multitude of human infections is due in part to an impressive array of extracellular and cell-wall associated virulence determinants that are coordinately expressed in this organism (51). The coordinate expression of many of these virulence determinants in *S.aureus* and other bacteria is regulated by global regulatory elements such as sarA (staphylococcal accessory regulatory protein A) and agr (15, 34). These regulatory elements in turn control the transcription of a wide variety of unlinked genes many of which have been implicated in pathogenesis.--